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Note

High-performance liquid chromatographic determination of iohexol in plasma, urine and feces

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Iohexol, 5-[acetyl(2,3-dihydroxypropyl)amino]-N,N′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide, Fig. 1, is a new non-ionic radiographic contrast agent which has sufficient stability for delivery to the X-ray laboratory as a sterilized solution. Like others of this class of diagnostic agents [1], it is not metabolized, but excreted in an unchanged form [2]. This paper describes a specific high-performance liquid chromatographic (HPLC) method for the quantitation of iohexol in spiked samples of human plasma, urine and feces.

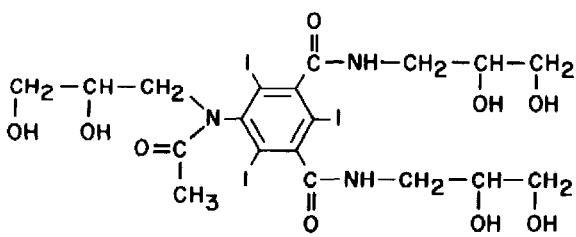

Fig. 1. Structural formula for iohexol.

EXPERIMENTAL

Chemicals

Iohexol and the internal standard for the assay, 5-acetylamino-2,4,6-triiodo-N,N′-bis-(2,3-dihydroxypropyl)-1,3-benzenedicarboxamide, were synthesized at the Sterling-Winthrop Research Institute. Other chemicals were obtained commercially (reagent grade) and used without further purification.

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Analysis of plasma samples

To a tube containing 1.0 ml of spiked plasma, 50 μl of internal standard solution (250 μg/ml in water) were added. The tube was mixed and a 100-μl aliquot was transferred to a clean tube and mixed with 850 μl of water, 50 μl of 0.2 *N* perchloric acid and 5 ml of chloroform. After centrifugation, a 400-μl aliquot of aqueous phase was transferred to a clean tube and mixed with 38 μl of 1.0 *N* sodium hydroxide. The sample was filtered with a centrifugal filtration apparatus (Bioanalytical Systems MF-1, W. Lafayette, IN, U.S.A.). A 100-μl aliquot was injected into the HPLC system. A set of standards, in duplicate, covering the range of zero and 2.8—27.8 μg/ml was prepared; both standards and samples were chromatographed under the following conditions: mobile phase, 0.01 *M* sodium phosphate buffer, pH 7.4—methanol (96:4, v/v); flow-rate, 1.75 ml/min; retention times, internal standard 4.9 min and iohexol 6.0 min.

Analysis of urine samples

Internal standard (150 μl of 3 mg/ml in water) was added to 850 μl of iohexol-containing urine. After mixing, a 50-μl aliquot was added to 2450 μl of water (1:50 dilution). A portion of the diluted sample was centrifugally filtered. A duplicate set of standards, covering the range of zero and 133—2670 μg/ml, was prepared. All samples were chromatographed under the following conditions: mobile phase, 0.01 *M* sodium phosphate buffer, pH 7.4—methanol (94:6, v/v); flow-rate, 1.75 ml/min; retention times, internal standard 4.4 min and iohexol 5.2 min.

Analysis of fecal samples

A fecal homogenate was prepared by adding 4 ml of iohexol-containing ethanol to 1 g of feces in a Waring blender. Internal standard (100 μl of 1 mg/ml in water) was added to a 1.0-ml aliquot of fecal homogenate. Ethanol, 10 ml, was added to each sample and the mixture was centrifuged. The supernatant was decanted into a clean silanized tube and dried in a 40°C water bath under a stream of nitrogen. The residue was dissolved in 1.35 ml of 0.05 *N* sodium hydroxide and passed through a 3.8 cm × 6 mm I.D. column packed with 50—100 mesh Dowex 50W-X4 resin (Na^+). The column was rinsed with 1.65 ml of 0.05 *N* sodium hydroxide. The combined eluate was adjusted to pH 3.8 with 1.5 *N* hydrochloric acid. The sample was centrifugally filtered and a 100-μl aliquot was injected onto the HPLC system. A set of standards, in duplicate, covering the range of zero and 125—2500 μg/g of feces, was prepared. Samples and standards were chromatographed under the following conditions: mobile phase, 0.01 *M* sodium phosphate buffer, pH 7.4—methanol (95:5, v/v); flow-rate, 1.5 ml/min; retention times, internal standard 8.5 min and iohexol 11 min.

HPLC system

The pump was a Constametric II (Laboratory Data Control, Riviera Beach, FL, U.S.A.) high pressure pump; the injector was a Rheodyne (Cotati, CA, U.S.A.) syringe-loaded valve; and the detector was an Altex (Berkeley, CA, U.S.A.) Model 153 UV detector with a 254-nm wavelength filter. The column

was a 5-μm Spherisorb ODS, Excalibar (Applied Science, State College, PA, U.S.A.), 25 cm × 4.6 mm I.D. column. Between the pump and the injector was a wide-bore precolumn, packed with bulk silica, which saturated the mobile phase with silica as a precautionary measure to protect the analytical column. A guard column (Waters, Milford, MA, U.S.A.) packed with glass beads was placed between the injector and the analytical column. The dimensions of the precolumn and guard column are not critical. The HPLC system was used at ambient temperature.

Data processing

The HPLC detector was interfaced with a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 3354 Laboratory Automation System which computed peak heights and peak height ratios, iohexol:internal standard, for each standard and sample. A least-squares regression analysis was performed and the linear regression model was used to determine the concentration of iohexol in the sample by inverse prediction [3]. The minimum quantifiable level (MQL) of the assay was estimated as that concentration at which the lower 80% confidence interval just encompassed zero [4].

The observed concentrations for the prepared, spiked samples were expressed as percent differences from the nominal values. The range of these percent differences was used to define the accuracy of the assay. Precision was estimated from the square root of the mean square error term (standard deviation) derived from the analysis of variance on percent differences.

RESULTS AND DISCUSSION

Plasma samples

Representative chromatograms of treated plasma are shown in Fig. 2 (left half). The assay was linear over the range of standards employed, 0 and 2.8—27.8 μg/ml.

The concentrations of the prepared plasma samples are summarized in Table I. The accuracy of the method, defined as the mean percentage difference from the nominal value, ranged from −3.2% to +5.3%. The estimated precision of the assay, from the square root of the mean square error term of the two-way analysis of variance, was ±4.2%. The mean (±S.E.) MQL was 0.54 (±0.23) μg/ml.

Urine samples

Fig. 2 (right half) shows representative chromatograms of processed urine standards. The assay was linear over the range of standards, 0 and 133—2670 μg/ml. The results of the analysis of the prepared urine samples are summarized in Table I. The urine samples at the lowest concentration were found to vary by more than 20% from the nominal concentration; they were reanalyzed against standards in the range of 0 and 66.7—667 μg/ml. Under these conditions, the mean percent differences were −1.59% and −2.54% for the 147 and 380 μg/ml samples, respectively, which were acceptable. The MQL obtained with the 66.7—667 μg/ml standards was 10.2 μg/ml; the mean (±S.E.) MQL from the full sets of standards was 26.5 (± 1.1) μg/ml. Truncating the

TABLE I

CONCENTRATIONS OF IOHEXOL IN PREPARED PLASMA, URINE AND FECAL SAMPLES

Plasma		Urine		Feces	
Nominal concentrations (μg/ml)	Assayed concentration (μg/ml)	Nominal concentrations (μg/ml)	Assayed concentration (μg/ml)	Nominal concentrations (μg/ml)	Assayed concentration (μg/ml)
0	<MQL*	0	<MQL**	0	<MQL***
	<MQL		<MQL		<MQL
	<MQL		<MQL		<MQL
3.1	3.1	147	135	138	133
	3.4		135		133
	3.1		136		131
Mean	3.2		135		132
S.E.M. (%)	3.1		0.25		0.50
Mean percent difference	3.2		−7.94		−4.11
5.7	6.0	380	378	263	254
	6.1		382		258
	5.7		379		254
Mean	5.9		380		255
S.E.M. (%)	2.0		0.32		0.52
Mean percent difference	4.1		−0.09		−2.92
15.3	15.5	971	990	540	543
	16.5		996		538
	15.2		1010		549
Mean	15.7		999		543
S.E.M. (%)	2.5		0.59		0.59
Mean percent difference	2.8		2.85		0.62
20.0	20.1	1800	1810	1430	1500
	20.6		1810		1460
	20.7		1830		1450
Mean	20.5		1820		1470
S.E.M. (%)	0.9		0.37		1.04
Mean percent difference	2.3		0.93		2.80
27.2	27.0	2400	2400	2300	2280
	27.9		2390		2290
	29.1		2440		2250
Mean	28.0		2410		2270
S.E.M. (%)	2.2		0.63		0.53
Mean percent difference	2.9		0.42		−1.16

*Mean MQL = 0.54 μg/ml.
**Mean MQL = 26.5 μg/ml.
***Mean MQL = 15.9 μg/g.

set of standards, which are run in duplicate, reduces the variability in the standard line; this results in lower estimated MQL values for the urine assay and reduces the mean percent differences for the samples containing low concentrations of iohexol. The accuracy of the urine assay ranged from −7.94% to 4.36% with an estimated precision of 1.35%.

Fecal samples

Representative chromatograms of processed fecal homogenates are shown in Fig. 3. The assay was linear over the range of 0 and 125—2500 μg/g of feces.

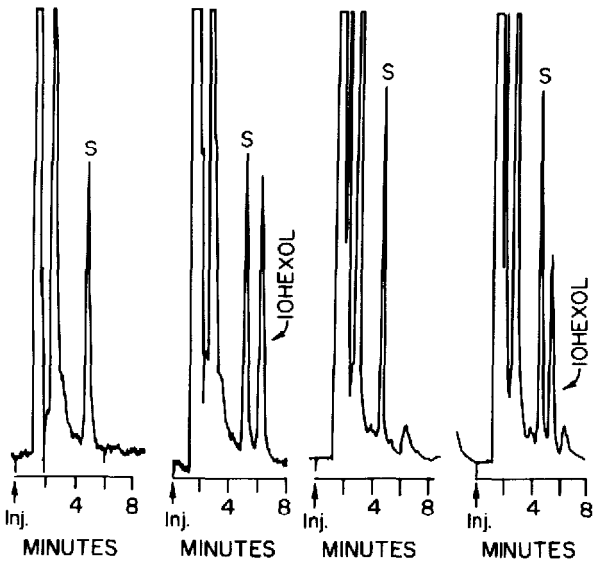

Fig. 2. Chromatograms of control human plasma (left half) containing the internal standard, S, and the same sample containing 16.7 μg/ml of iohexol. Chromatograms of control human urine (right half) containing the internal standard and the same sample containing 400 μg/ml of iohexol. Tallest peaks are full scale. Attenuation 0.01 a.u.f.s.; chart speed 0.5 cm/min.

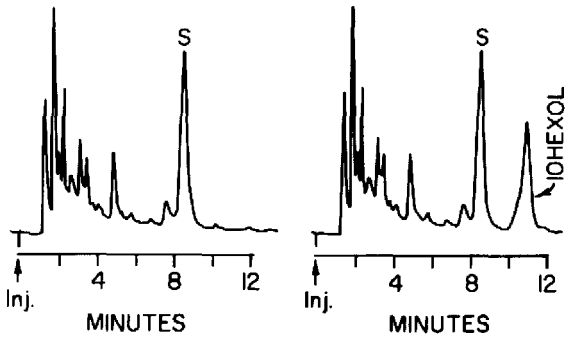

Fig. 3. Chromatograms of a processed fecal sample containing (left panel) the internal standard, S, and (right panel) the same sample containing 500 μg of iohexol, per g of feces. The internal standard peak is 18% of full scale. Attenuation 0.16 a.u.f.s.; chart speed 0.5 cm/min.

The observed concentrations for the prepared samples are shown in Table I. The accuracy ranged from −4.11% to +3.77%, with an estimated precision of ±1.13%. The mean (±S.E.) MQL was 15.9 (±3.70) μg/g of feces.

The extraction efficiencies of iohexol and the internal standard from feces, based on a comparison of peak heights of extracted versus direct standards, were 83.8% and 87.3%, respectively. Since neither plasma nor urine were subjected to an extraction procedure, recovery data were not needed.

Small changes in the composition of the mobile phase and the flow-rate are necessary to avoid the presence of small interference peaks. These can be seen in the urine samples (Fig. 2, right half), eluting after the iohexol peak and in the fecal samples (Fig. 3) eluting just before the internal standard.

Sets of spiked samples that had been stored, in the frozen state, for periods

up to 30 days were also analyzed. The assay results were not significantly different from the results that were obtained with samples that were analyzed immediately after preparation.

In summary, an accurate, selective, reproducible and precise HPLC assay has been developed for the measurement of iohexol concentration in human plasma, urine and feces. This method has proven useful for the analysis of specimens obtained during clinical trials; these results will be reported elsewhere.

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